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Investigation 7: Cell Division
Part B: Meiosis and Crossing Over

Background

Ascomycota are a diverse group of fungi including the familiar single-celled baker's yeast, the complex morel mushroom, and the deadly *Aspergillus flavus*. In fact, 75% of all fungi are grouped as Ascomycota. Geneticists have altered one particular species of Ascomycota, *Soardaria fimicola*, for use in studying crossing over during meiosis.

Crossing over occurs during prometaphase I of meiosis. During crossing over, homologous pairs of chromosomes exchange sections of DNA that contain the same genes. Therefore, the exact genotype of the new offspring will vary from that of its parents (see Figure 1). It is important to note that crossing over does not have to occur during each generation, nor does it always take place at the same point of exchange. Over time, however, crossing over leads to a greater variety of genes in a population and contributes to a diversity of characteristics and an overall stronger population. This strength is then reflected in the ability of the population to adapt to changes in the environment and also to evolve.

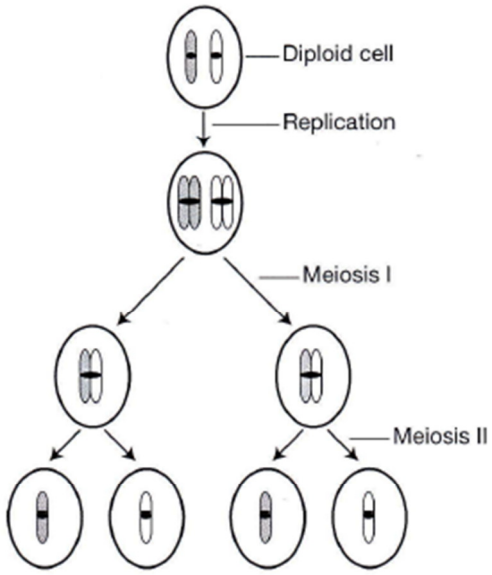

Figure 1.

Meiosis involves two cellular divisions, meiosis I and meiosis II. In meiosis I the chromosomes condense, replicate, crossover, and divide in two. In meiosis II, the chromosomes do not replicate again. Instead each chromosome is split in half through the centromere leaving one copy of each gene in each haploid cell. In the fungi kingdom, meiosis occurs in specialized fruiting bodies. In the group *Ascomycota* this specialized fruiting body is called an *ascocarp* or *perithecium*. The frequency of crossing over is studied in genetics because it allows scientists to map genes and estimate the distance between two genes or between a gene and the centromere of the chromosome. The daughter cells are called *ascospores* or, in more general terms, *spores*. The daughter cells are all contained within a single tube-like structure called an *ascus* (plura = *asci*). The structure and properties of the ascus make *Sordaira fimicola* useful for studying crossing over.

Many *Ascomycota*, like *S. fimicola*, spend most of their time as haploid cells. Numerous clone copies of each haploid cell unite to form thread-like hyphae. Small holes between cell walls allow the sharing of nutrients and water between the cells of each hypha. Masses of hyphae intertwine to form mats of fungi. One of the reasons that fungi spread so easily is that these haploid hyphae are able to break off and generate a new organism anywhere nutrients are available. *S. fimicola* grows on rotting vegetation or dung in the wild making it a common mold in the environment. If no nutrients are

available, the fungus is able to form haploid spores using asexual reproduction or sexual reproduction. The spores can be dispersed in the wind or settle into the soil until conditions improve. In the lab, *S. fimicola* is easily cultured on agar plates.

During sexual reproduction hyphae of different haploid *S. fimicola* come into contact allowing cells in the hyphae to fuse and form a single cell with two nuclei, one from each individual. This fused cell is called a *dikaryon*. The dikaryon is not considered diploid since the two nuclei are from separate fungi and the nuclei are not fused together. The dikaryon cells undergo multiple rounds of mitosis to form a mass of cells. This mass of cells can exist for years without undergoing fusion of the nuclei. Sexual reproduction occurs when some of the dikaryon nuclei fuse. After fusion the fruiting body forms and meiosis occurs, creating the asci and ascospores of the next set of haploid cells.

The ascospres form inside the tight confines of the tube-like asci. The ascospores actually line up in order based upon which cell produced that particular ascospore. In 1956, a geneticist named Lindsay S. Olive (1917-1988) published an article about crossing over in *S. fimicola*. Dr. Olive used ultraviolet light to cause mutations in the genes of *S. fimicola*. After numerous trials Dr. Olive produced a mutation in the gene that produces the pigment in the ascospore. The production of the black pigment is either greatly reduced or completely repressed in the mutated strain of *S. fimicola*. A reduction in the amount of black pigment results in gray spores. An absence of black pigment results in tan ascospores. By collecting the gray or tan ascospores Dr. Olive was able to produce true breeding fungi much like Mendel's peas.

Collecting the ascospores is easy because the fruiting body produced by *S. fimicola* is shaped like a vase (see Figure 2). The vase-shaped *perithecium* is produced on a dikaryon stalk above the dikaryon mass of cells. Within the perithecium each ascus lines up with the top opening of the perithecium. The ascospores are ejected out of the opening into the wind for dispersal. Wet-mount microscope slide preparations of the perithecium result in the asci spreading out like spokes on a wheel, lining up for analysis. The distance between the centromere and the gene that codes for the black pigment can be determined by counting the ascospores with a population of asci. This distance is called the *map distance* and is reported or measured in terms of *map units*. A map unit is an arbitrary unit of measurement where one map unit corresponds to 1% crossover. The likelihood of crossover occurring between two genes on the same chromosome increases as the distance between the genes increases. Similarly, a gene is more likely to crossover if the gene is not adjacent to the centromere of the chromosome. By definition, the number of map units between two genes or between the gene and the centromere is equivalent to the percent of genes that undergo crossover. In order to count the number of crossing over events a culture of wild type (black) *S. fimicola* and a culture of tan mutant *S. fimicola* are grown adjacent to each other in a culture dish.

Figure 2.

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Recall that each ascospore can be tracked back to the parent chromosome. The pattern of black and tan ascospores shows whether crossing over occurred during meiosis. Observe Figure 3. Note that the diagram of the asci indicates eight ascospores in each ascus, not the expected four cells. With *S. fimicola* each of the four haploid daughter cells undergoes a single mitosis after the end of meiosis II. So each daughter cell produces a clone of itself. These clones reside next to each other within the ascus. If the cells come from parents with identical pigment genes the ascus will contain eight spores that are the same color whether black or tan. If the cells come from parents with each pigment type but crossing over did not occur, the spores will appear as four black wild-type and four tan mutant spores (4b:4t). If crossing over between a black wild-type and a tan mutant occurred during meiosis I, the four spores will have one of two possible patterns. Patterns of 2:2:2:2 and 2:4:2 are possible. Each of the numbers can be either tan or black. This is written out as 2b:2t:2b:2t or 2t:2b:2t:2b and 2b:4t:2b or 2t:4b:2t.

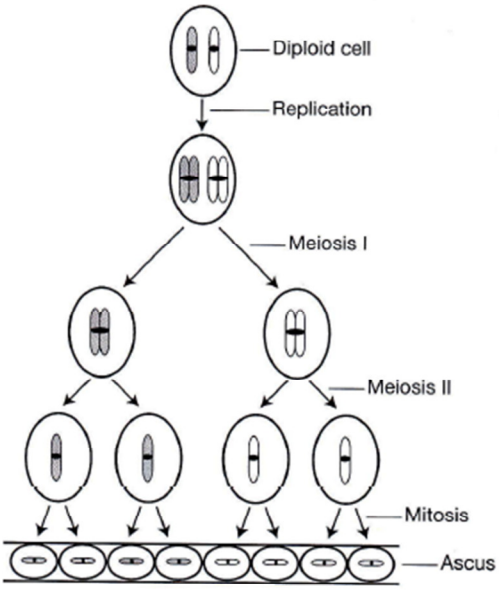

Figure 3a. Noncrossing over asci—4b:4t

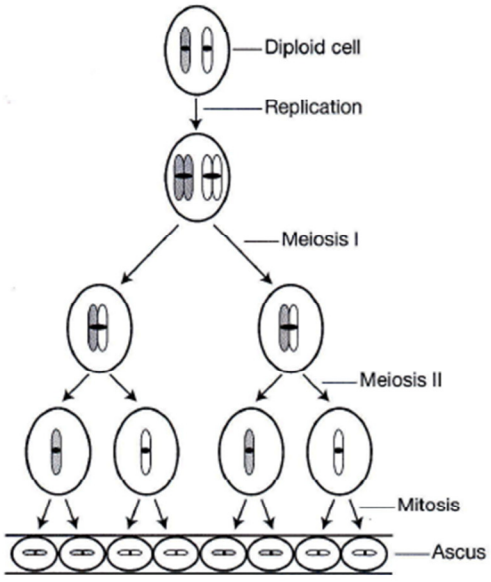

Figure 3b. Crossover asci—2b:2t:2b:2t

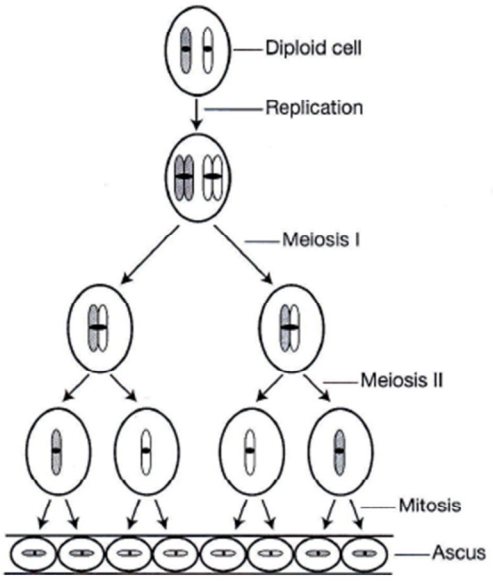

Figure 3c. Crossover asci—2b:4t:2b

Purpose

The purpose of this genetics simulation activity is to identify the phenotype for each ascospore within a hybrid ascus and to count the number of asci corresponding to each type of crossover and noncrossover genotype.

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Pre-Lab Questions

1. In order to calculate the number of map units between the centromere and the gene, at least 100 heterozygous asci are counted. Why is it necessary to count so many asci?

2. The drawings in Figures 3a-c show only heterozygous asci. What would homozygous asci look like?

3. Why will we only be categorizing heterozygous asci and not homozygous ones?

4. Why are map unit calculations important to geneticists?

5. What does a high percentage of crossover indicate in terms of the distance between two genes (or in this case, their distance from the centromere)?

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Procedure

1. Begin with one of the five *Sordaria* cards assigned. Categorize each heterozygous ascus starting from the center of the card and working your way out to the edge.
    - Don't forget which asci you already counted! (There are 20 per card)
    - Record the number of the card above the data table
2. Place the tally mark in Table 1 corresponding to the correct genotype for each ascus.
    - Note: The possible genetoypes are:
        - Non-crossover
            - ✓ 4b:4t
            - ✓ 4t:4b
        - Crossover
            - ✓ 2b:2t:2b:2t
            - ✓ 2t:2b:2t:2b
            - ✓ 2b:4t:2b
            - ✓ 2t:4b:2t
    - See Figures 3a-c for schematics of possible hybrid asci
3. Categorize all 20 of the hybrid asci on the first card.
4. Add up the tally marks for each genotype counted on that card and record in the "Total" column.
5. Record the total number of non-crossover vs crossover asci for that card.
6. Repeat steps 1-5 with the four remaining cards (record data in Tables 2-5).
7. Record your totals from all five cards in Table 6
8. Complete the calculations and questions under the analysis section.

Results

Table 1: Crossover vs Non-crossover
Counts for Card #____

Genotype	Tally Marks	Total
4b:4t		
4t:4b		
Non-crossover Asci		
2b:2t:2b:2t		
2t:2b:2t:2b		
2b:4t:2b		
2t:4b:2t		
Crossover Asci		

Table 2: Crossover vs Non-crossover
Counts for Card #____

Genotype	Tally Marks	Total
4b:4t		
4t:4b		
Non-crossover Asci		
2b:2t:2b:2t		
2t:2b:2t:2b		
2b:4t:2b		
2t:4b:2t		
Crossover Asci		

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Table 3: Crossover vs Non-crossover Counts for Card #____

Genotype	Tally Marks	Total
4b:4t		
4t:4b		
Non-crossover Asci		
2b:2t:2b:2t		
2t:2b:2t:2b		
2b:4t:2b		
2t:4b:2t		
Crossover Asci		

Table 4: Crossover vs Non-crossover Counts for Card #____

Genotype	Tally Marks	Total
4b:4t		
4t:4b		
Non-crossover Asci		
2b:2t:2b:2t		
2t:2b:2t:2b		
2b:4t:2b		
2t:4b:2t		
Crossover Asci		

Table 5: Crossover vs Non-crossover Counts for Card #____

Genotype	Tally Marks	Total
4b:4t		
4t:4b		
Non-crossover Asci		
2b:2t:2b:2t		
2t:2b:2t:2b		
2b:4t:2b		
2t:4b:2t		
Crossover Asci		

Table 6: Crossover vs Non-crossover TOTALS

	Total From All 5 cards
Non-crossover Asci	
Crossover Asci	
Total Asci Counted	100

Analysis

We can use the frequency (proportion) of crossover-produced ascospores as a measure of the relative distance separating the gene locus and the centromere. Here, the recombinant frequency would be equal to the number of recombinant (crossover) asci per total asci counted (both recombinant and non-recombinant).

$$\text{recombinant frequency} = \frac{\text{recombinant asci}}{\text{total asci}} \times 100$$

1. Calculate the recombination frequency using the correct values from Table 6 and the equation above. Be sure to show your work.

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Geneticists define a crossover map unit as the distance on a chromosome that produces one recombinant post-meiotic product per 100 post-meiotic products. The distance is given in the units of centi-Morgans (cM). Given that map units express the percent recombinant asci resulting from crossovers and each single crossover produces 4 recombinant spores and 4 nonrecombinant spores (so only 4 out of the 8 are 'different'), the map unit distance for our Sordaria cross is always **one half** the frequency of crossing over for the gene.

$$\text{map units} = \frac{\text{recombinant frequency}}{2}$$

2. Determine the map distance between the gene for spore color and the centromere using the equation above. Show your work. Include units in your final answer.

3. Was the number of each type of crossover phenotype observed relatively constant? (ie: # of 2b:2t:2b:2t vs # of 2t:2b:2t:2b vs # of 2b:4t:2b vs # of 2t:4b:2t)
EXPLAIN why you would expect this.

4. A similar technique can be used to determine the distance between two genes on a single chromosome. In this lab, a color mutation was used as the gene of interest. What is the benefit for using a color mutant gene for learning about map units?